BULLETIN OF THE
U.S. DEPARTMENT OF AGRICULTURE
No. 118.

Contribution from the Bureau of Entomology, L. O. Howard, Chief.
July 14, 1914.

(PROFESSIONAL PAPER.)

EXPERIMENTS IN THE DESTRUCTION OF FLY LARVÆ IN HORSE MANURE.

By F. C. Cook, *Physiological Chemist, Bureau of Chemistry*, R. H. Hutchison, *Scientific Assistant, Bureau of Entomology*, and F. M. Scales, *Assistant Mycologist, Bureau of Plant Industry.*

INTRODUCTION.

The great activity in antifly campaigns in recent years, together with the recognition of the fly as a disease carrier, has created such widespread demand for some means of destroying the fly that this investigation has been undertaken for the purpose of finding a chemical that would destroy this pest in its principal breeding place, namely, horse manure, without injuring the bacteria or reducing the fertilizing value of the manure. This work was undertaken in 1913 at the suggestion of Dr. L. O. Howard to Dr. C. L. Alsberg, who has heartily cooperated in this study and secured the cooperation of Dr. W. A. Taylor. The entomological work was done under the direction of Mr. W. D. Hunter and the bacteriological work in cooperation with the laboratory of Mr. K. F. Kellerman. It is the purpose of this paper to review some recent experiments, the results of which point to an economical, practical, and effective way of destroying fly larvæ by the chemical treatment of manure. A consideration of the larvicidal powers of a number of chemicals more or less effective as larvicides, together with an account of their effects on the value of manure so far as may be estimated by chemical and bacteriological analyses, is included.

HISTORICAL.

American workers were the first to attack the problem of the chemical treatment of manure with a view to destroying fly larvæ. Pioneer work of this nature was begun in 1897 by Dr. L. O. Howard, who showed that kerosene emulsion, while effective with small

amounts of manure, was not practical for use on a large scale. Chlorid of lime, however, was found to be a good maggot killer, but its action on the bacteria was not studied. Dr. Howard (1911)[1] published an account of his own experiments and of the work of other investigators.

Prof. S. A. Forbes (Howard, 1911, p. 197), State entomologist of Illinois, found that lime, borax, borax and sodium arsenate mixture, iron sulphate, and carbon bisulphid—the last in closed-box tests—were effective larvicides.

Herms (1910) claims that many of the common insecticides are more or less effective if used in proper concentrations and amounts, but none of these can be applied with safety, as they are poisonous, inflammable, or corrosive.

In 1912 Prof. R. I. Smith (Smith, 1912, p. 64), then State entomologist of North Carolina, found that 2 gallons of kerosene sprinkled over 25 square feet of a manure pile gave no indication of any larvicidal action. Acid phosphate proved entirely worthless from the standpoint of killing the maggots, even when used at the rate of 400 pounds to every 2,000 pounds of manure. Finely ground phosphate rock (floats) had no effect on the larvæ. A 4 per cent formaldehyde solution thoroughly applied to heavily infested manure piles did not destroy any maggots.

This seems to be the extent of the experimental work, as reported in the literature, up to the year 1913. It is evident that the chemical treatment of manure has not received the attention which it deserves. Moreover, Dr. Howard (1911) has pointed out that all these experiments have left unanswered the question as to what effect the treatment will have on the manure itself. No analyses were made to determine how the chemical composition of the manure was affected by the larvicides; nor were any field experiments carried out to ascertain whether the fertilizing value of the manure was altered in any way.

MANURE: ITS RÔLE IN FLY BREEDING.

As stable manure is one of the most valuable fertilizers known, a large number of investigations have been carried on to determine the best means of utilizing as well as preserving it. In addition to its content of nitrogen, phosphorus, and potash the value of manure depends on the number and species of bacteria present, as well as on its content of organic material which the bacteria convert into plant food. Manure, when undergoing fermentation in the open, loses some of its valuable nitrogenous constituents, especially ammonia and

[1] Authors and dates in parentheses refer to "Literature cited," p. 26.

gaseous nitrogen, the extent of the loss depending on the nature of the fermentation, the aerobic fermentation, due to the rapidity of combustion, producing a greater loss than the anaerobic. To prevent this loss of plant food in the course of fermentation, various chemicals have been used, either to retard bacterial action or to fix the volatile constituents. Among the various substances used for this purpose may be mentioned ground phosphate rock (floats), kainit, various lime compounds, carbon bisulphid, formaldehyde, and ferrous sulphate.

The house fly is attracted to horse manure, possibly by its odor, and on alighting crawls an inch or so under the surface and there lays its eggs. On account of the temperature of the manure the eggs hatch within one day. The larval or maggot stage continues from four to five days, during which the larvæ migrate to the sides of the pile and toward the base, feeding on the manure during their journey. The pupæ are found, after a few days, congregated in the outer edges of the manure near the ground, as seen in Plate I. It is therefore about 10 days from the time the eggs are laid until the mature fly emerges.

GENERAL PLAN OF EXPERIMENTAL WORK.

Experiments were carried out at the Experimental Farm of the Bureau of Plant Industry at Arlington, Va., and continued during the autumn at the Experiment Station at Audubon Park, New Orleans, La., under a cooperative arrangement entered into by the Bureau of Entomology, the Bureau of Chemistry, and the Bureau of Plant Industry.

CAGE EXPERIMENTS.

An idea of the structure of the 15 cages, which were designed by Mr. W. D. Pierce, of the Bureau of Entomology, may be gained from the accompanying photograph (Pl. II). Each cage has an inside measurement of 2 by 2 by 4 feet. The bottom of the cage consists of a galvanized-iron pan 1 foot high. Above this pan bronzed wire screening (16 meshes to the inch) is tacked both on the inside and outside of the framework. These two layers of screening are 2 inches apart. In this way manure once put into the cages was protected from further infestation from the outside. In order to prevent the larvæ from escaping from the sides of the cages through this screening it was found necessary to fasten sheets of tin on the inside above the galvanized-iron base. These strips are 1 foot high, and thus there was afforded a space of 8 cubic feet from which larvæ had little chance to escape. In the bottom of the cage nine small holes were made which permitted excess liquids to drain off. Some larvæ found their way out through them, but these were caught in the pan below and a record kept of the numbers thus escaping.

The top of the cage is a wooden door which is fastened down tightly with hinges and hasps. In the center of this door is an opening 5 inches in diameter and above this a board provided with two openings of the same size. Cone-shaped flytraps are fitted into these openings. This board is placed in grooves so that either one of the two traps may be brought over the opening in the door by merely sliding the board.

On one side of the cage is a small trapdoor 5 inches square through which samples of manure may be taken out for examination.

Each cage stands on legs 4 inches high and in a galvanized-iron pan 3 feet square with sides 4 inches high. This pan serves to collect drip water and escaping larvæ, and to isolate the cage from such predatory insects as ants.

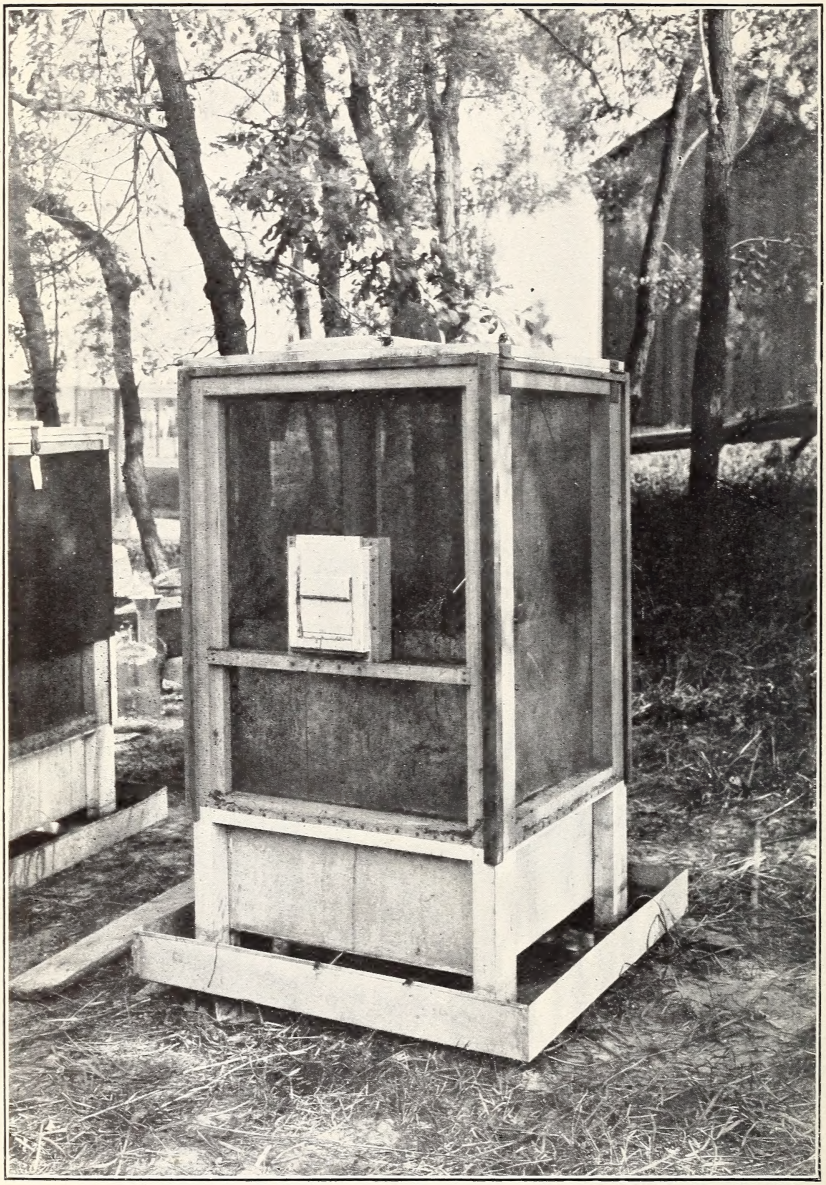
Eight bushels of manure were used in each of the cage experiments. It was dumped in at the top and the chemical, in solution, was sprinkled on with a watering can. After two preliminary experiments it was found necessary, in order to insure thorough penetration, to use 10 gallons of the liquid per 8 bushels; that is, at the rate of 1 gallon to 1 cubic foot. Usually the sprinkling was done in three layers by putting 2 bushels of manure in the cage and applying $2\frac{1}{2}$ gallons of the solution. This was repeated in the second layer of 2 bushels. Finally, the remaining 4 bushels were added and the last 5 gallons of the solution applied. When a chemical was applied in dry condition it was scattered over the surface of the manure, which was treated in three layers as in the case of the solution; 10 gallons of water were afterwards added.

The manure in the control cages was sprinkled with water equal to the volume of the solutions of the chemicals used. In this way the moisture content of the manure was made as nearly as possible the same in all cages. It will be understood that 10 gallons of solution were applied to 8 bushels of manure in all the cage experiments mentioned below, unless some other explanation is given. After treatment in this way the doors of the cages were closed and the flytraps put in place. The cages were examined every day. The escape of any larvæ into the drip pan was noted, and the volume of the drip water measured and a sample analyzed. A quart sample of manure was removed through the small door at the side of the cage after a day or two and the percentage of living and dead maggots determined. The larval counts of quart samples were very unsatisfactory so far as indicating the comparative larvicidal value of the chemicals, but the results of some of these counts are given in the tables.

After five to seven days flies began to emerge, and then it was necessary to darken the cages with black cloth tacked on the sides, as seen

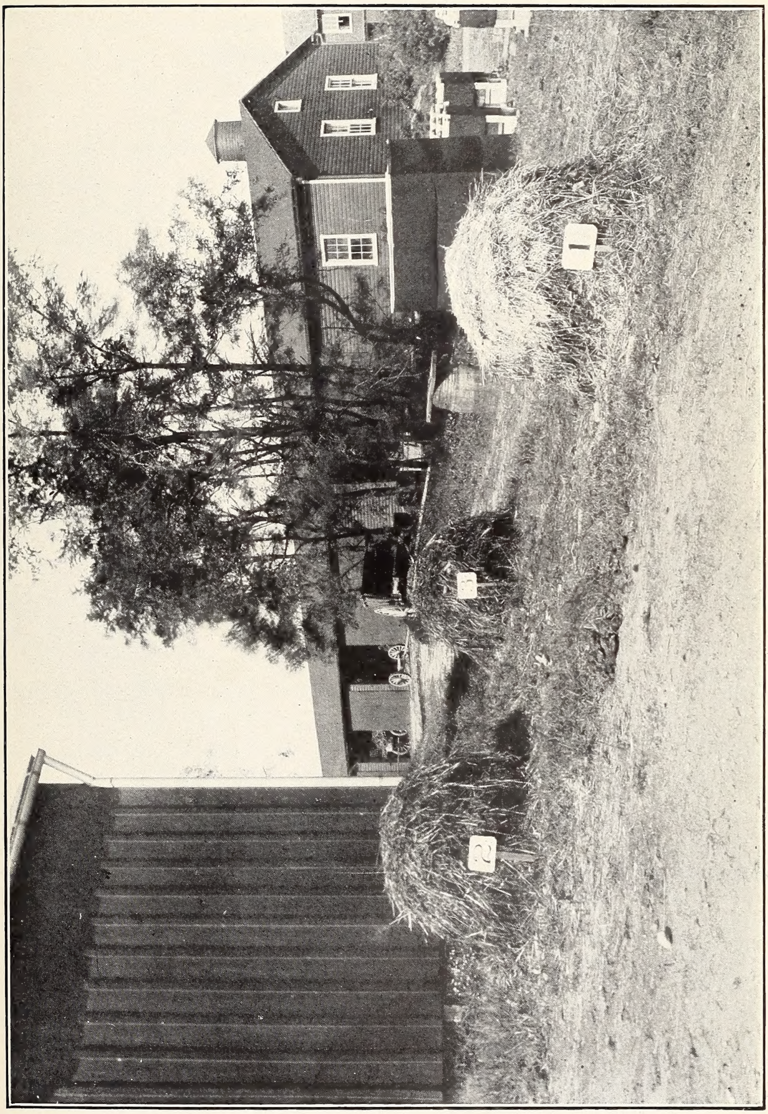
DESTRUCTION OF FLY LARVÆ IN HORSE MANURE.

View of manure pile cut in half. The places where the fly pupæ are found, just above the ground and around the edges of the pile, are indicated by the two pieces of white cardboard. (Original.)



DESTRUCTION OF FLY LARVÆ IN HORSE MANURE.

Cage used in the chemical treatment of manure, showing the flytraps at the top, the small door at the side through which samples of manure can be removed, the pan for collecting drip water, and other details of the structure. (Original.)



DESTRUCTION OF FLY LARVÆ IN HORSE MANURE.

A view of some open-pile experiments to show the size of the piles and the method of treatment. No. 1 has been treated with dry powdered borax, No. 2 with a solution of borax, and No. 3 is the control pile, which is sprinkled with water only. (Original.)

on the cage to the left in Plate II. In this way the only light came from the opening into the flytrap at the top, and flies very soon after emerging made their way up into the trap. The flies caught in the traps were chloroformed and counted daily. At the end of each experiment the total numbers of flies from each cage were compared. The difference between the total numbers of flies from a cage of treated manure and from the control cages is taken as an index of the effectiveness of the chemical. In any one set of experiments the manure used was all from the same source and, being in fresh condition, contained only eggs and larvæ. It was mixed before transferring to the cages, but it is evident that under the conditions we could not be sure of an equal infestation in all cages. Therefore the chemicals were not regarded as having any larvicidal power if the differences in the totals were small.

OPEN-PILE EXPERIMENTS.

In order to simulate natural conditions a parallel series of experiments was carried out by treating manure piles on the ground. Here again 8 bushels were used for each treatment, but repeated applications of both manure and chemicals were made. At the beginning of an experiment a quantity of fresh manure was divided into piles of 8 bushels each. Chemicals to be tested were tried at the rate of 10 gallons to 8 bushels except as otherwise noted. One pile was sprinkled with water only and was used as a control. On the following day another lot of fresh manure was similarly divided and piled on top of that of the previous day, and the treatment repeated. At the end of four days there was a pile of 32 bushels which had received four applications of chemicals. Plate III gives an idea of the size of the piles and shows that the experiments were carried out on a practical scale.

Eight to ten days after the fourth and last treatment the piles were opened and gone over carefully in search of pupæ. The pupæ were collected from the edges of the piles (compare Pl. I), spread on a large sheet of paper, counted, and the numbers compared. Chemical and bacteriological examinations were made of certain of these open piles.

METHODS OF SAMPLING.

Manure consists of urine and dung more or less intimately mixed with straw, wood shavings, sawdust, peat, or other absorbent. When first carried from the stable it is not uniform in composition, as the dung may predominate in one part of the mass and the straw or other absorbent in another part. Thorough mixing will help greatly in making it more uniform, but as the eggs and larvæ in the manure

are readily shaken out, it can not be mixed as thoroughly as desired, and consequently there is no way under ordinary field conditions by which a small sample may be obtained that will be truly representative.

The errors due to sampling are necessarily large, and the differences in the results from the controls show the extent of this variation. This is unavoidable and must be recognized in all work on manure, and applies to the bacteriological results as well as to the chemical data, but is not so pronounced in the former cases, as the difference between the counts of the controls and treated samples is so much greater than for the chemical results.

In order to secure the most uniform samples under these conditions for bacteriological and chemical analyses, the following procedure for obtaining samples was adopted. Approximately an inch of material was first removed from the top and then half a pound of the underlying manure weighed on a spring balance; another half pound was then weighed from the center of the pile, and finally the same quantity was taken from the bottom. The three samples were all put in the same container for transportation to the laboratory, where the whole sample was spread out on a clean sheet of wrapping paper and then cut into small pieces and thoroughly mixed. When the material appeared quite uniform the sample was quartered. One quarter was then cut into half-centimeter lengths with clean shears. The straw or shavings were cut with the other material. When this was completed the sample was again thoroughly mixed. As the bacterial content of manure is very high, no attempt was made to work under absolutely sterile conditions because the contamination arising from ordinary handling of the material was of no importance when compared with the great number of organisms present. However, precautions were taken to prevent excessive contamination by using clean paper, shears, etc., for each sample. The carefully prepared quarter sample was put in a clean Mason jar.

BACTERIOLOGICAL EXAMINATION.

Two 10-gram samples of the manure, prepared as described above, were taken for each bacteriological determination. A sterile spatula was used to convey the sample from the jar to the tared watch glass on the balance pan. One of the 10-gram samples was dried at 100° C. for one hour to determine the percentage of solids. The other sample was brushed into a 2-liter flask containing 1 liter of sterile water. The cotton plug was thereupon replaced by a clean rubber stopper which had been lightly flamed. The flask was then vigorously shaken for five minutes and again, after a five-minute interval, for three minutes. A 1 c. c. sample was then withdrawn and run into 100 c. c.

of sterile water. Five dilutions were prepared, ranging from 1 part in 10,000 to 1 part in 100,000,000. A duplicate series of Petri dishes was then prepared from these dilutions and standard beef agar. After five days' incubation at 28–30° C. the plates were counted. The average counts of the duplicate plates were taken and converted into equivalents for 1 gram of dry manure by the use of the figures obtained from the duplicate 10-gram samples that had been dried at 100° C.

The results obtained by plating on the standard beef agar are comparative and serve to show the germicidal action of the chemicals on the majority of the bacteria present in the manure. The total bacterial counts on this medium include not only some of the bacteria that increase the value of the manure by their metabolic processes, but also many that may decrease its value in the same way by destroying nitrogen salts available for plant food. For this reason the total bacteriological counts on beef agar are not considered as entirely indicative of the fertilizing value of the manure. It is even possible that the germicidal effect of formaldehyde, calcium cyanamid, and potassium cyanid in the manure might prove highly beneficial, as Russell and Buddin's (1913) results with formaldehyde, toluene, cresol, phenol, etc., in the soil indicate.

CHEMICAL EXAMINATION.

The method of taking the samples was described above, but the samples for chemical examination were twice run through a sausage grinder after cutting with shears and were placed in screw-capped Mason jars provided with rubbers and analyzed as soon as possible. Samples for chemical examination were taken from the control cages immediately after the experiments were started, and from all 15 cages after 10 days. In this way it was thought an idea of the change which had taken place in the various samples could be obtained, the changes in the controls being taken as an index of the normal rate of decomposition of the manure.

The manure samples were analyzed for solids, ash, ammonia, and nitrogen, using the methods of the Association of Agricultural Chemists (Wiley, 1908). The total nitrogen determinations were made by the nitrogen laboratory of the Bureau of Chemistry. The results obtained by the magnesium oxid distillation method for ammonia, although much higher, showed the same general tendencies as the results obtained on the water extracts.

Water extracts of the manure were prepared from each sample by taking 25 grams of the finely divided manure and adding 500 c. c. of distilled water, allowing them to stand for one hour, with occasional shaking. The solutions were filtered through S. & S. folded filters No. 588, and the following determinations were made: Water-

soluble nitrogen, ammonia, amino nitrogen, nitrites, nitrates, and reaction.

Ammonia was extracted by the Folin and Macallum (1912) aeration method and nesslerized. The amino nitrogen was determined by the Van Slyke method (Van Slyke, 1911), but as very little nitrogen in this form was present in the extracts, the figures are not given. Nitrites were determined with the sulphanilic acid reagent and nitrates by the reduction method with aluminum foil (American Public Health Association, Laboratory Section, 1912). Nitrites and nitrates were not usually found in the samples examined, because the manure had not stood sufficiently long. The reaction was determined by taking 20 c. c. of the water extract, diluting with 200 c. c. of carbon dioxid free water, and titrating with N/20 acid, using Alizarin red as indicator. Fehling's solution was not reduced by any of the 20 or more water extracts tested.

GENERAL ACCOUNT OF CHEMICALS USED.

In the course of the season 24 different chemicals were tried in various concentrations. Of these only seven have shown any effective larvicidal action in the strengths used. In the following paragraphs some of the chemicals which gave negative results are first noted, and later in the paper those which appeared to have the greatest value are described in more detail.

CHEMICALS WHICH GAVE LOW LARVICIDAL RESULTS.

KEROSENE EMULSION.

Kerosene emulsion, prepared according to the Riley-Hubbard standard formula, was used in strengths varying from 1 part emulsion in 5 parts water to 1 part emulsion in 50 parts water. In no case were results obtained which showed any appreciable larvicidal action. Even from the cage subjected to the strongest dosage 956 flies were taken, the average from the two control cages being 1,355 flies.

No chemical analyses of the manure were made. The bacterial count, where the strongest emulsion (1–5) was used, was 16,600 million per 1 gram of dry manure as compared with 6,130 million in the controls. These counts were made eight days after treatment with the chemical, but as the bacterial content of manure varies greatly and only one determination was made no conclusion can be drawn.

Kerosene emulsion was not used on any open-pile experiments. We have already called attention to the fact that Dr. Howard in his tests found that this reagent was ineffective when applied on a large scale.

KAINIT.

Kainit, which consists of potassium chlorid and magnesium sulphate, furnished us by Dr. F. Zerban, of New Orleans, was used in two cage experiments and in one open-pile test. In the cage experiments 4 pounds of kainit were used. The total number of flies obtained from the treated cages averaged 2,194, and from the two controls 3,104 flies. In the open-pile experiment three applications of 4 pounds per 8 bushels were made, and after 10 days about 12,000 pupæ were found. The corresponding control pile contained about 20,000 pupæ.

In the two cage experiments no chemical or bacteriological examinations were made. In the open-pile experiment the bacterial count was high, 17.5 million, as compared with 5.9 million in the control. One hundred c. c. of water extract, equivalent to 5 grams of the manure, from the treated pile contained a trace of nitrites and nitrates. No nitrites or nitrates were found in the kainit, nor did the control manure show any. The ammonia nitrogen in the kainit-treated manure was 12.3 per cent and in the control manure but 8.8 per cent of the total nitrogen. The high bacterial count and the increased amount of NH_3 obtained, as well as the fact that nitrates were found in the kainit-treated and not in the control manure, suggests that this compound may have a stimulating action on the bacteria, but no conclusions are justified from this one test. This chemical may be used to reenforce manure, but possesses little larvicidal power.

PYROLIGNEOUS ACID.

Pyroligneous acid was used in commercial form without dilution. Certain claims have been made in some districts of the South, especially in North Carolina, that pyroligneous acid is of value as a repellent, and in our experiments special attention was given to this point. Two piles of fresh manure of 8 bushels each were sprinkled with 10 gallons of pyroligneous acid. Before treatment no eggs were to be found anywhere on the surface of either pile. Two hours later fresh batches of eggs were found on both piles. The pupæ collected numbered about 6,000 and 8,000. Further observations showed that fly eggs were deposited on other piles of manure treated with the pyroligneous acid. Evidently the pyroligneous acid has little, if any, value as either a repellent or a larvicide. The bacterial counts showed a great increase, rising from 25 million in the control to 653 million in one of the pyroligneous acid piles.

ISTHMIAN CANAL COMMISSION'S LARVICIDE.

The Isthmian Canal Commission's larvicide, which has been successfully applied in the Canal Zone for the purpose of killing mosquito larvæ, is prepared according to the following formula: 150

gallons of carbolic acid are heated to 212° F. and to this 150 pounds of finely broken resin and 30 pounds of caustic soda are added and the mixture kept at 212° F. till a dark emulsion without sediment is formed. The resultant emulsion is a good larvicide, 1 part to 10,000 parts of water killing mosquito larvæ in less than half an hour. However, we did not find it effective against house-fly larvæ. The results of three cage experiments are given in Table I, Series A, Nos. 1, 2, and 3. Compared with the corresponding controls (Nos. 7, 8, and 9) it seems as if few, if any, fly larvæ were destroyed, but the fact that a considerable number of larvæ were found in the drip water from the control and only a few from the three treated cages should be considered.

The chemical analyses, given in Table I, show variation in the total nitrogen of the treated and control manures. This is true of many of the samples analyzed and shows the normal variations. The water extract of the treated manure showed more nitrogen and ammonia present than did the water extract of the control manure. The reactions of the water extracts varied considerably. No nitrites or nitrates were present either in the larvicide treated or in the control manure.

Unfavorable action on the bacteria is shown where the numbers are progressively decreased as the volume of the larvicide was increased. The highest count for the larvicide-treated samples is considerably lower than the lowest control count.

Several open-pile experiments were also carried out. One of these was started September 15 and the treatment repeated on four successive days. From the resulting pile of 32 bushels of manure about 10,000 pupæ were taken on September 26. The control pile contained about 7,000 pupæ. This was a typical experiment and is sufficient to show that even with repeated daily applications this reagent is of no value as a maggot destroyer.

IRON SULPHATE.

The results of three cage experiments with iron sulphate are given in Table I, Series A, Nos. 4, 5, and 6. The controls for these are Nos. 7, 8, and 9. The total number of flies caught from these cages shows that the manure was rather lightly infested. However, a comparison of the total number of flies that emerged and the number of larvæ found in the drip pan from treated and untreated cages indicates that this chemical may have had some larvicidal power. However, in three other cage experiments not shown in the table no larvicidal action was evidenced.

Iron sulphate was not used on open piles. The chemical and bacteriological findings in Table I show an injurious action on the

manure. The number of bacteria was noticeably reduced, varying inversely with the strength of the solution used. The amounts of water-soluble nitrogen were materially lowered in the iron-sulphate-treated manure, depending on the amount of iron sulphate employed. The iron sulphate evidently acts as a precipitant for some of the water-soluble nitrogen compounds. The ammonia was fully doubled, due possibly to the reduction of alkaline reaction, two of these three samples showing a faint acidity. Iron sulphate blackened the manure and deodorized it, as noted by Forbes. On the whole, we find iron sulphate less effective as a larvicide than Forbes's experiments seem to indicate. It is important, however, to note that the amount of iron sulphate used by Forbes was much greater than that used in these tests.

TABLE I.—*Destruction of fly larvæ in horse manure—Results with ineffective larvicides—Cage experiments at Arlington, Va., summer of 1913.*

No.	Treatment of 8 bushels of manure; 10 gallons used whenever solution was applied.	Larval mortality, 1 quart sample of manure 2 days after treatment. Alive.	Larval mortality, 1 quart sample of manure 2 days after treatment. Dead.	Flies emerged.	Larvæ killed.	Larvæ in drip pan.	Bacteria per 1 gram of manure, dried at 100° C.	Manure, total nitrogen.	Water extract. In per cent of total nitrogen. Nitrogen.	Water extract. In per cent of total nitrogen. Ammonia nitrogen.	Water extract. Alkalinity, N/20 H_2SO_4 per 100 c. c. (5 grams of manure).
Series A:				*Number.*	*Per cent.*	*Number.*	*Millions.*	*Per cent.*	*Per cent.*	*Per cent.*	*C. c.*
1	Canal larvicide, 1-75 (7½ gallons)	0	5	113	0	6	3,700	0.73	35.62	6.58	12.00
2	Canal larvicide, 1-75 (10 gallons)	0	6	110	0	1	2,600	.61	34.43	3.93	5.50
3	Canal larvicide, 1-75 (12½ gallons)	......	......	179	0	0	1,600	.53	32.08	3.96	5.75
4	Iron sulphate, 1½ pounds per gallon	2	0	73	32.4	0	700	1.05	10.48	5.05	1.50
5	Iron sulphate, 1 pound per gallon	6	1	171	0	0	970	.67	16.42	6.72	[1] 0.62
6	Iron sulphate, ½ pound per gallon	1	0	81	25.0	0	2,800	.76	22.37	6.84	[1] 1.25
7	Control (water only)	32	0	146	0	15	5,200	.84	26.19	2.62	10.50
8	......do	22	0	102	0	127	6,000	.68	25.00	3.09	6.50
9	......do	5	0	76	0	221	5,100	.65	18.46	2.46	5.00
Series B:											
1	Sodium chlorid, 2½ pounds per gallon	28	1	141	55.5	0	2,550	.51	32.94	7.65	4.40
2	Sodium chlorid, 1 pound per gallon	110	0	217	30.0	100	....	.45	28.67	3.78	7.50
3	Copper sulphate, 1 pound per gallon	5	2	101	67.4	0	648	.69	9.71	3.78	2.75
4	Copper sulphate, ½ pound per gallon	4	0	132	57.4	Few.	4,070	.75	14.93	2.40	7.75
5	Control (water only)	48	0	322	0	100	3,060	.55	23.45	2.55	7.75
6	......do	12	0	298	0	30	4,800	.72	21.11	2.08	7.50

[1] Acidity.

SODIUM CHLORID (TABLE SALT).

The results of two cage experiments with manure treated with sodium chlorid are given in Table I, Series B, Nos. 1 and 2. The corresponding control cages are numbered 5 and 6. The average

number of flies from these two controls is 310. Presuming that the infestation of the manure at the start of the experiment was the same in all cages, it appears from the table that sodium chlorid used at the rate of $2\frac{1}{2}$ pounds per gallon killed 55 per cent of the larvæ. The 1-pound per gallon application showed a 30 per cent destruction of the maggots. The chemical results of the salt-treated manure are not very different from those of the untreated manure except that there is an apparent increase in the nitrogen and ammonia in the water extract of the treated samples. Only one bacterial examination was made and this showed that the strongest salt solution reduced the number of bacteria somewhat.

COPPER SULPHATE.

Nos. 3 and 4 of Series B, Table I, give the results of two cage experiments with copper sulphate. When compared with the controls it would seem that the dosage of 1 pound per gallon killed 67 per cent of the maggots and the one-fourth pound strength 57 per cent.

The bactericidal power of copper sulphate is well known. When added at the rate of 1 pound per gallon sufficient copper sulphate remained in solution to kill 87 per cent of the bacteria. Their number was not affected by the smaller quantity of this chemical.

The chemical analyses show an injurious effect from the heavier application of copper sulphate, which reduced the amount of soluble nitrogen and the alkaline reaction of the water extract. With the weaker strength the only apparent effect is a slight reduction of water-soluble nitrogen. No open-pile experiments with copper sulphate were carried out.

LIME-SULPHUR MIXTURE.

Lime-sulphur was used in three cage experiments, but in no open piles. There is no evidence that the lime-sulphur possessed any larvicidal power, for more flies developed from the cage receiving a 1–5 treatment than from the control. The bacteria do not appear to be affected by this treatment. From two other experiments where lime-sulphur was used in strengths of 1–15 and 1–30 fewer flies emerged than in the control, but this was probably due to differences of infestation.

In addition to the chemicals mentioned, acid phosphate, a proprietary fertilizer, and several proprietary disinfectants were tested with negative larvicidal results.

PARTIALLY EFFECTIVE LARVICIDES.

In Table II, page 15, some results obtained with potassium cyanid, Paris green, and formaldehyde, which were found to possess some larvicidal action, are recorded. Each of these three substances in the

heaviest application, and formaldehyde in all cases, reduced the number of bacteria.

POTASSIUM CYANID.

Potassium cyanid gave favorable results in three cage experiments. These results are given in Table II, Series C, Nos. 1, 2, and 3, the control being No. 4. Quart samples of manure two days after treatment showed a large percentage of dead larvæ for the two stronger applications. The total numbers of flies developing were very much reduced. It appears that the two higher concentrations killed 93 per cent of the larvæ. The chemical results of analyses of these three samples of manure show considerable variations, but there is no evidence that the manure had been injured by the application of the potassium cyanid. The increased alkalinity results of the control and of No. 2 may be explained by the large amount of water-soluble nitrogen in these two cases. No open piles were treated with potassium cyanid. This reagent, when used in proper concentrations, will undoubtedly be found a very effective maggot killer, but its extremely poisonous nature makes it objectionable and dangerous. The bacterial counts show that potassium cyanid in the manure had no very definite bactericidal effect. A stimulating action is rather indicated in the two higher dilutions, but as the difference in the number of bacteria between the three treated samples is no greater than that between some of the controls, no conclusions can be drawn from this experiment.

PARIS GREEN.

Paris green was used in three cage experiments, the results of which, together with those of the corresponding controls, are given in Table II, Series D. The Paris green was not all dissolved, but was applied in the form of a suspension. The suspended particles were deposited on the surface and only the part in solution filtered into the deeper parts of the manure. It appears from these experiments that Paris green killed from 70 to 90 per cent of the larvæ.

The bacteriological counts vary considerably and inversely with the strength of the solution used. The most concentrated solution was strongly bactericidal and reduced the number of organisms by about 50 per cent. The higher dilutions showed the general stimulating action of poisons in small quantities. The effect in general is the same as that of potassium cyanid, but is much more marked.

The water-soluble nitrogen varied with the amount of Paris green used, and was lowest where the strongest application of Paris green was made, due probably to the precipitating power of the copper, and about equal to the control where the two weaker applications were made.

FORMALDEHYDE.

Formaldehyde solution was used in six cage experiments, but on no open piles. Three concentrations were tried, by mixing 1 part of the commercial 40 per cent formalin with 3, 6, and 12 parts of water, respectively. The results of three of these tests are given in Table II, Series E, together with the corresponding controls. In three experiments not given in the table the infestation of the manure was so slight that it was not possible to form any judgment as to the larvicidal action of this chemical. Even in the experiments which are given in the table, the manure was lightly infested. However, all the concentrations show considerable larvicidal action. Taking the average total number of flies of the controls it is evident that from 75 to 85 per cent were killed. It is probable that if this treatment had been made in closed boxes or receptacles to retard the loss of formaldehyde by evaporation, the larvicidal action would have been still higher.

As might be expected, the formaldehyde in these dilutions caused a great reduction in the number of bacteria. The highest dilution (1–12) killed 99.6 per cent of the bacteria that would grow on beef agar. The chemical results show a decreased alkalinity of the water extract. The ammonia results average slightly higher than those obtained on the control samples, but in No. 2, where the dilution of formaldehyde used was 1–6, the bacterial count, the water-soluble nitrogen, the ammonia, and the alkalinity are higher than in either of the other two treated samples. The fact that formaldehyde produces an acid reaction, either by conversion to formic acid or by combining with amino acids, a reaction used by Sörensen (1907) for the quantitative estimation of the amino acids, may explain the reduced alkalinity of these extracts. Nitrites and nitrates were detected in all three cases of the manure treated with formaldehyde. It is interesting in this connection to note that Russell and Buddin (1913) carried out some experiments on the action of various volatile antiseptics in the soil, and found that formaldehyde increased the production of nitrates and ammonia. While formaldehyde is extremely disagreeable to work with on account of the irritating action which it has on the mucous membrane, nevertheless further work with this chemical will be undertaken.

TABLE II.—*Destruction of fly larvæ in horse manure—Results with partially effective larvicides—Cage experiments at Arlington, Va., summer of 1913.*

No.	Treatment of 8 bushels of manure; 10 gallons used whenever solution was applied.	Larval mortality, 1 quart sample of manure 2 days after treatment.		Flies emerged.	Larvæ killed.	Larvæ in drip pan.	Bacteria per 1 gram of manure, dried at 100° C.	Manure, total nitrogen.	Water extract.		
									In per cent of total nitrogen.		Alkalinity, $N/20\ H_2SO_4$ per 100 c. c. (5 grams of manure).
		Alive.	Dead.						Nitrogen.	Ammonia nitrogen.	
				Number.	*Per cent.*	*Number.*	*Millions.*	*Per cent.*	*Per cent.*	*Per cent.*	*C. c.*
Series C:											
1	Potassium cyanid, 0.1 per cent solution.	2	9	82	93.6	......	5,250	0.68	19.85	3.09	10.25
2	Potassium cyanid, 0.02 per cent solution.	11	21	86	93.3	100	7,260	1.00	23.60	2.90	14.50
3	Potassium cyanid, 0.004 per cent solution.	11	4	251	80.6	350	7,620	.63	20.48	Trace.	10.00
4	Control (water only)	64	1	1,287	0	400	6,130	1.12	24.11	3.57	17.65
Series D:											
1	Paris green, 1–20	0	2	92	70.3	Few.	1,740	.70	13.43	2.71	9.00
2	Paris green, 1–40	......	......	35	88.7	Few.	7,300	.59	22.88	1.86	7.50
3	Paris green, 1–80	0	1	32	89.7	Few.	19,950	.56	25.00	4.64	6.00
4	Control (water only)	48	0	322	0	100	3,060	.55	23.45	2.55	7.75
5	....do	12	0	298	0	30	4,800	.72	21.11	2.08	7.50
Series E:											
1	Formaldehyde,[1] 1-3 solution.	0	7	20	81.5	1	14	.58	18.97	3.62	.75
2	Formaldehyde, 1–6 solution.	3	1	16	85.2	22	44	.46	21.74	4.57	2.00
3	Formaldehyde, 1–12 solution.	165	15	27	75	0	22	.60	18.33	2.83	.75
4	Control (water only)	32	0	146	0	15	5,200	.84	26.19	2.62	10.50
5	....do	22	0	102	0	127	6,000	.68	25.00	3.09	6.50
6	....do	5	0	76	0	221	5,100	.65	18.46	2.46	5.00

[1] Nitrites and nitrates were found in Nos. 1, 2, and 3, Series E.

SODIUM FLUORID.

Sodium fluorid was used in two cage experiments. In one it was applied at the rate of 2 pounds per gallon, and 454 flies developed. In the other 1 pound per gallon was used, and 1,053 flies developed. From the two control cages the totals were 6,152 and 5,870. Thus the stronger concentration destroyed over 90 per cent of the maggots, and the weaker strength 84 per cent. No open piles were treated.

No bacteriological or chemical analyses were made of the manure treated with sodium fluorid. From the limited number of tests with this chemical, it is evident that it may possess some value as a larvicide, and further experiments will be conducted, using commercial sodium fluorid, although the cost (5 pounds, $1) may prohibit its general use.

AMMONIACAL GAS LIQUOR.

Ammoniacal gas liquor, which is a by-product of the manufacture of illuminating gas, evidenced some larvicidal effect when used in the strengths of 1–5 and 1–25. From the cage treated with the stronger dosage 206 flies were caught and 179 flies from the

other. The control cages showed 1,508 and 1,287 flies. The gas liquor in the 1–5 strength was strongly bactericidal, reducing the number of bacteria as shown in the control from 6,130 million to 92.8 million. In view of the fact that the gas liquor showed a bactericidal action and that the transportation of a liquid in large amounts is expensive, it was not studied further, although it possesses certain advantages, as it contains a considerable amount of nitrogen, practically all of which is in the form of ammonia. This nitrogen is, however, all in soluble and volatile form and easily lost.

CALCIUM CYANAMID.

The treatment with calcium cyanamid was tried at the suggestion of Dr. Alsberg. It has been used in cage experiments at Arlington, Va., and the results obtained are recorded in Table III.

TABLE III.—*Destruction of fly larvæ in horse manure—Larvicidal results with calcium cyanamid—Cage experiments at Arlington, Va., summer of 1913.*

No.	Treatment of 8 bushels of manure with 10 gallons of water.	Larval mortality, 1 quart sample of manure 2 days after treatment.		Flies emerged.	Larvæ killed.	Larvæ in drip pan.
		Alive.	Dead.			
Series F:				*Number.*	*Per cent.*	*Number.*
1	Calcium cyanamid, 20 pounds	1	2	7	99.5	0
2	Calcium cyanamid, 5 pounds	0	4	52	96.3	....
3	Control	22	0	1,508	0	12
4	.....do	64	1	1,287	0	400
Series G:						
1	Calcium cyanamid, 5 pounds	4	0	92	20.0	30
2	Calcium cyanamid, 4 pounds	4	1	761	....	20
3	Calcium cyanamid, 3 pounds	....	....	56	51.3	25
4	Control	82	0	25	0	50
5	.....do	22	0	204	0	10

The calcium cyanamid was scattered over the manure in powdered form and in all cases water was added. From the table it appears that the 20-pound application killed over 99 per cent of the larvæ. The 5-pound applications gave varying results, as seen in the table, and in one cage experiment not shown 58 per cent of the larvæ were destroyed. This gives an average larvicidal power of 58 per cent for this amount of the calcium cyanamid. In one cage test not shown where 4 pounds were applied, 40 per cent were killed, but in the cage experiment given in Table III no larvicidal action was apparent. Since calcium cyanamid is used to some extent as a fertilizer and is a means of adding nitrogen to the manure, and thus to the soil, it is highly desirable that a further study of this chemical be made, not only to determine more exactly its larvicidal action, but also to de-

DESTRUCTION OF FLY LARVÆ IN HORSE MANURE.

On the left larvæ are shown which have been killed by borax. They were in the process of changing to pupæ. On the right normal pupæ are seen. (Original.)

termine by field experiments whether the amount of nitrogen thus added compensates for the cost of treatment. The cost of the cyanamid in 100 or 200 pound lots is about 3½ cents per pound.

The results of two typical open-pile experiments with calcium cyanamid are given in Table IV. The 5-pound application killed 82 per cent of the larvæ and reduced the number of bacteria markedly. The 4-pound application killed 71 per cent of the larvæ and reduced the bacteria 50 per cent. In both cases the water-soluble nitrogen, ammonia, and alkalinity were considerably increased.

TABLE IV.—*Destruction of fly larvæ in horse manure—Results with calcium cyanamid—Open-pile experiments (three applications) at New Orleans, La., November, 1913.*

No.	Treatment of 8 bushels of manure with 10 gallons of water.	Total number of pupæ found after 8 to 10 days.	Larvæ killed.	Bacteria per 1 gram manure, dried at 100° C.	Manure.		Water extract.		
							In per cent of total nitrogen.		Alkalinity, N/20 H_2SO_4 per 100 c. c. (5 grams manure).
					Solids.	Total nitrogen.	Nitrogen.	Ammonia nitrogen.	
			Per cent.	*Millions.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*C. c.*
Series H:									
1.......	Calcium cyanamid, 5 pounds...	3,500	81.6	43	31.30	0.72	44.44	8.89	7.35
2.......	Calcium cyanamid, 4 pounds...	5,500	71.0	75	30.47	.59	47.46	13.56	8.15
3.......	Control........................	19,000	0	158	27.14	.43	19.54	6.51	5.30

EFFECTIVE LARVICIDES (BORATES).

The most favorable results were obtained by the use of borax (sodium borate) and calcined colemanite (crude calcium borate). Both substances possessed a marked larvicidal action and appeared to exert no permanent injury on the bacteria. These two borates have been used in a large number of experiments and the results all uniformly show a very high larvicidal action, both in cages and open piles, and whether applied in dry form or in solution.

A comparison of the total number of flies or of pupæ from borax-treated manure with the totals from control manure shows a larvicidal power of over 99 per cent in nearly all trials. One of the reasons why borax is so effective in reducing the number of flies is due to its toxic effect on the eggs, which do not hatch after contact with this chemical. The piles in one experiment, started on September 13, 1913, were examined for pupæ on September 25. At this time large masses of eggs of the house fly, perhaps 600 to 800, were found in a borax-treated pile. They were not empty, collapsed shells, but had normal shape and evidently had not hatched. They were somewhat discolored, many having a bluish tinge. Some of these were

taken to the laboratory and examined daily under a microscope. None of these hatched after a week at room temperature and favorable moisture conditions. On October 6, in going over a pile, last treated with borax solution on September 28, batches of a thousand eggs or more were found. They had a bluish tinge. A mass of these eggs with surrounding manure was kept in a jar in the laboratory for a week and examined daily. None had hatched at the end of this time. Similar observations were made on other borax-treated piles. No such masses of unhatched eggs were ever found on control piles, nor on piles treated with other chemicals after the first three or four days of exposure.

Calcined colemanite, being largely insoluble, did not show this effect on the eggs. Borax acts very effectively through its toxic action on the eggs, but its action is not confined to the egg stage, as larvæ are also killed. In nearly all cases examinations of open piles showed the presence of dead larvæ as well as pupæ. In Table V it will be noted that in some piles large numbers of pupæ were found, but these were black, shrunken, wrinkled, and were not normal in shape, having more nearly the form of the larvæ than of the pupæ. Pl. IV.) When kept in the laboratory for a long time 1 per cent or less hatched. The borax had evidently killed them just at the time of transformation from larvæ to pupæ. This may be explained in several ways. (1) It may be that the larvæ, in the younger stages, resisted the action of the borax they had ingested but became very sensitive to it at the time of the breakdown of larval tissues. (2) The action of the borax may be cumulative and so may not evidence its toxic action until toward the end of the larval stage. (3) It may be that the larvæ in their earlier stages were found some distance in from the surface where the borax had not penetrated, but that, when ready to pupate, they migrated to the outer lower edges of the manure pile where the concentration of the borax was greatest and were killed by it. The migration of the larvæ in the cages and open piles has already been referred to on pages 3 and 5, and is discussed more in detail by Mr. Hutchison (1914).

The fact that small quantities of borax are not detrimental to the normal fermentation of manure is further shown by some temperature determinations.

The manure piles were made with no attempt to pack the manure, because it was believed that the higher temperatures prevailing where aerobic fermentation was in progress would be an attraction to the flies. Three series of experiments were used for these tests. The temperatures were taken by inserting a thermometer about a foot deep in the top of the piles. As the piles were small the temperatures at this depth were very nearly the maximum. The three controls attained their highest temperature, 66° and 67° C. (150.8° and 152.6°

F.) in from five to seven days after the experiment was started. At the same time the borax-treated piles reached their maximum of 58° to 63° C. (136.4° to 145.4° F.). Even where one-eighth pound of borax was used the temperature was slightly suppressed, as it reached only 61° C. (141.8° F.). This effect, however, may have been due to the borax preventing the growth of organisms which produce fire-fanging. The effect of borax in entirely preventing this condition has been reserved for a future investigation. However, it was found that in three cases the control piles showed evidences of firefanging and the presence of a white powdery mold in the interior. This condition was never found in the borax-treated piles. After attaining a maximum, the temperature of all the piles declined rapidly. The treated ones continued lower than the controls.

One manure pile treated with 5 pounds of calcined colemanite showed a steady decline in temperature from the beginning of the experiment. The bactericidal effect of this large dose is further shown by a comparison of the bacterial count obtained from a sample of this pile and that of the control; a decrease of 64 per cent in the number of bacteria occurred.

The data of the borax-treated manure are recorded in Tables V and VI. The open-pile experiments, which are recorded in Table V, show marked variations in numbers of bacteria, but whether this is due to a variation in the penetration of the borax because of different natural factors, or because the samples were not representative of the pile, although taken in the usual manner (see page 5), can not be stated at this time. There is a reduction in the number of bacteria in Series J, Nos. 1 and 2, and Series L, Nos. 1 and 2, where colemanite was used. There are marked increases in Series I, Nos. 1 and 2, and Series K, Nos. 3 and 4. In Table VI, where the results are recorded for the manure experiments made in cages, an increase in the number of bacteria is seen in all the borax-treated samples.

The manure from the open-pile experiments, Table V, indicates an increase of water-soluble nitrogen and ammonia in the borate-treated samples. The reaction of the water extract is increased in all of these cases. Further, in four of the open-pile experiments nitrites and nitrates were both found. In no case did the control manure give a reaction for nitrites or nitrates. The presence of nitrites and nitrates in the borax-treated piles is very interesting and if it is obtained in all cases where the borax-treated manure has been allowed to stand for several weeks a strong argument will be presented for its use in addition to the effective larvicidal action which it is seen to possess. There are considerable variations in the water-soluble nitrogen and ammonia results for the open-pile experiments as well as for the bacterial counts as noted on page 6.

TABLE V.—*Destruction of fly larvæ in horse manure—Results with borates—Experiments on open piles, New Orleans, La., November, 1913.*

No.	Treatment of 8 bushels of manure; 10 gallons used whenever liquid was added.	Number of applications.	Total number of pupæ found after 8–10 days.	Bacteria per gram manure, dried at 100° C.	Manure.		Water extract.		
							In per cent of total nitrogen.		
					Solids.	Total nitrogen.	Nitrogen.	Ammonia nitrogen.	Alkalinity, N/20 H_2SO_4 per 100 c. c., 5 grams manure.
Series I:				*Millions.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*C. c.*
1	Na-borate,[1] 2½ pounds dry (no water added)	4	[2] 5,000	141	39.59	0.60	36.67	7.33	11.05
2	do.[1]	4	[2] 4,200	172	39.14	.55	29.09	8.18	10.60
3	Control (water)	4	10,000	105	34.54	.46	30.43	8.69	5.90
Series J:									
1	Na-borate, 2 pounds dry (no water)	4	30	.659	42.51	.67	44.78	12.54	10.20
2	do	4	39	.577	43.29	.68	39.71	11.03	10.90
3	Control (water)	4	2,500	316.	42.43	.63	30.16	8.89	6.50
Series K:									
1	Na-borate in solution, ⅙ pound per gallon	3	[2] 985	2	36.69	.52	25.00	8.27	12.45
2	do	3	[2] 575	5	43.08	.56	21.43	7.50	7.85
3	Na-borate [1] in solution, ⅕ pound per gallon	3	[3] 1,700	38	34.72	.47	34.04	11.91	8.55
4	do.[1]	3	[3] 1,900	36	43.01	.53	26.42	11.13	7.35
5	Control (water)	3	20,000	6	34.04	.49	24.49	8.78	6.10
Series L:									
1	Calcined colemanite, 3 pounds plus water	3	[4] 2,600	38	30.58	.49	30.61	16.33	7.05
2	do	3	[4] 3,200	26	30.77	.44	31.82	9.09	7.20
3	Control (water)	3	19,000	158	27.14	.43	19.54	6.51	5.30

[1] Nitrites and nitrates present.
[2] Approximate. Of pupæ from borax-treated piles about 1 per cent hatch.
[3] Of all these only 10 flies emerged after many days in the laboratory.
[4] Abnormal in shape and color. Only 1 fly developed in sample of 500 pupæ.

TABLE VI.—*Destruction of fly larvæ in horse manure—Results with borax—Cage experiments at New Orleans, La., November, 1913.*

No.	Treatment of 8 bushels of manure with 10 gallons of liquid.	Larval mortality, 1-quart sample manure 2 days after treatment.		Total number of flies emerged.	Larvæ in drip pan.	Bacteria per 1 gram manure, dried at 100° C.	Total nitrogen in manure.	Water extract.		
								In per cent of total nitrogen.		
		Alive.	Dead.					Nitrogen.	Ammonia nitrogen.	Alkalinity, N/20 H_2SO_4 per 100 c.c. (5 grams manure).
Series M:						*Million.*	*P. ct.*	*P. ct.*	*P. ct.*	*C. c.*
1	Borax, ½ pound per gallon	5	0	15	12	7,392	0.51	33.33	9.41	19.50
2	do	2	0	18	15	3,003	.55	18.18	10.18	16.50
3	Borax, ¼ pound per gallon	35	0	38	3	7,452	.58	27.59	11.55	13.00
4	do	5	4	22	6	5,800	.58	37.93	10.86	13.75
5	Control (water)	82	0	25	50	2,204	.74	16.22	4.46	8.30
6	do	22	0	204	10	3,484	.84	14.29	1.79	7.50

In the cage tests, Table VI, the water-soluble nitrogen, ammonia, and reaction were lower for the controls than for the borax-treated

manure. The low water-soluble nitrogen and ammonia results of the controls may possibly be due to the unusual fermentation going on in these two samples, as indicated by the peculiar odor. The fact that, after grinding, the manure tended to cake or lump may have prevented the usual amount of material from going into solution. The bacterial counts in the cage experiments are higher than the controls, and also higher than those of the open piles. This is undoubtedly due to the artificial conditions of the cage experiments. The increase of water-soluble nitrogen, ammonia, and alkalinity has been found in all the borax-treated manure, both cage and open-pile tests, at Arlington and New Orleans.

In Table VII additional cage experiments showing the larvicidal action of borax, dry and in solution, and calcined colemanite with water, are recorded. Borax in small amounts, such as $1\frac{1}{4}$ pounds per 8 bushels of manure, destroyed 98 to 99 per cent of the maggots, and calcined colemanite, even when 2 pounds per 8 bushels of manure were used, showed the same percentage of larvicidal action.

TABLE VII.—*Cage experiments showing larvicidal action of borates on fly larvæ in horse manure.*

No.	Treatment of 8 bushels of manure; 10 gallons used whenever liquid was added.	Total number of flies emerged.
Series N:		
1	Na-borate, dry powder, $2\frac{1}{2}$ pounds (no water added)	12
2	Na-borate in solution, $\frac{1}{4}$ pound per gallon	1
3	do	2
4	Control (water)	6,152
5	do	5,870
Series O:		
1	Na-borate in solution, $\frac{1}{4}$ pound per gallon	5
2	do	13
3	do	68
4	Na-borate in solution, $\frac{1}{8}$ pound per gallon	46
5	do	50
6	Calcined colemanite, 4 pounds plus water	55
7	Calcined colemanite, 3 pounds plus water	165
8	Calcined colemanite, 2 pounds plus water	29
9	Control (water)	3,069
10	do	3,140

RECENT EXPERIMENTS TO DETERMINE MINIMUM AMOUNTS OF BORAX AND CALCINED COLEMANITE WHICH ARE EFFECTIVE AS LARVICIDES.

Some recent tests at New Orleans to determine the minimum amounts of borax and calcined colemanite which are effective have shown that 0.62 pound of borax and 0.75 pound of calcined colemanite are effective as larvicides, but when smaller amounts of either are used their larvicidal value is reduced. It is therefore apparent that 0.62 pound of borax and 0.75 pound of calcined colemanite to 8 bushels of manure (10 cubic feet), with the addition of 2 to 3 gallons of water, are the minimum quantities of these borates that will destroy practically all the fly maggots in manure.

ADVANTAGES AND COST OF BORAX.

The great demand for borax, due to its uses in the arts and in the household, has made this substance available in all parts of the country. It has the further advantage of being comparatively nontoxic, noninflammable, and easily transported and handled, as it is a powder. Thus borax is superior to most of the substances that have been tested as larvicides. Several investigators (see Haselhoff, 1913) have shown that in small amounts borax has a stimulating effect on plant growth, while larger amounts are toxic.

Borax is prepared from colemanite (calcium borate), which is mined in California, and has the following composition: Boron trioxid, 50.9 per cent; calcium oxid, 27.2 per cent; water, 21.9 per cent. The crude colemanite was tested for its larvicidal action, but this was so slight, undoubtedly due to its insolubility, that it was discarded in favor of borax and calcined colemanite. Calcined colemanite is prepared from crude colemanite by simply subjecting it to high temperatures.

The crude colemanite is not sold as such, but a considerable amount of the calcined colemanite is used in various industries. The calcined colemanite is a gray powder and is largely, but not entirely, insoluble in water. It costs about 2 cents per pound in large shipments, and in smaller amounts sells at approximately 4 cents per pound. Borax ($Na_2B_4O_7 10H_2O$) is prepared from colemanite by treatment with soda ash. It retails at about 10 cents per pound, but can be obtained in 100-pound lots or more in Washington at 5 to 6 cents per pound. Borax is readily soluble in water.

EFFECTS OF BORAX-TREATED MANURE ON PLANTS.

The chemical analyses and bacterial counts to which references have been made throughout this bulletin do not indicate any permanent deleterious effects of the borax on manure. On the contrary, a beneficial effect is suggested. This was especially the case with the chemical results where an increase of ammonia was obtained in all cases and no apparent reduction in the total nitrogen was evident. Nitrites and nitrates were found in several of the open piles where borax had been applied. In order to be certain of the effect of borax-treated manure on plants, extensive experiments have been performed both in the greenhouse and in open plats. The field work was conducted at four points in the South, as well as on the Arlington farm, and the pot tests were conducted in the greenhouses of the department at Washington. The following plants were tested: Wheat, tomatoes, peas, beets, radishes, kohl-rabi, oats, corn, cucumber, lettuce, as well as apple seedlings and rosebushes. Such elaborate experiments seem to be necessary on account of the known toxic

effects of large applications of boron upon the growth of plants, as shown by several investigators. In this connection it is important to note that investigations of Russell and Buddin (1913) in England have shown that the application of very small amounts of volatile and some nonvolatile disinfectants have eventually resulted in the stimulation of plant growth. This same effect is indicated in some of the experiments with borax.

In the field and pot experiments no deleterious effects were observed from the application of borax at the rate of 0.62 pound per 8 bushels (10 cubic feet) of manure, except possibly on wheat. Larger doses of borax produced a discoloration of the tips of some other plants. In our field experiments with winter wheat the plants when 4 inches high showed a decided yellowing of the tips where very heavy applications of borax were made, but at the start of the growing period in the spring the yellowing of the tips decreased and the wheat was nearly normal in appearance. These effects vary with the plants and the amount of moisture present in the soil. Where rainfall is heavy the effects disappear quickly. At Orlando, Fla., for instance, where the experiment was conducted during a drought and larger amounts of borax than 0.62 pound per 8 bushels were used, injurious effects were much more evident than in other localities. In all these cases, however, except at Orlando, recent observations have shown that the plants have practically recovered—so far as can be determined without estimating the actual yields, which can not be done at the present time. From these experiments it is believed that no injurious effects will follow the application of the minimum amount of borax found necessary to destroy the larvæ, namely, 0.62 pound per 8 bushels of manure, which may be applied to the field at the rate of 15 tons per acre. If more is necessary, untreated manure may be used. Some recent pot tests have indicated that the addition of slaked lime in amounts equal to half that of the borax present tends to offset the toxic action which results from heavy applications of borax. Some questions relating to the effects of borax on the growth of plants remain to be determined, notably its possible cumulative action, and these will be reported later. It is expected that interesting results will follow from the experiments now under way with calcined colemanite, which, though cheaper than borax, is effective in destroying fly larvæ when applied at the rate of 0.75 pound per 8 bushels.

SUMMARY.

CLASSIFICATION OF CHEMICALS TESTED.

The substances used in the experiments dealt with in this bulletin may be arranged in two classes, as indicated below. The term "satisfactory" is used to indicate destructive action on fly larvæ,

noninjurious effect on manure, and lack of extremely poisonous properties. Among the unsatisfactory or partially satisfactory substances are included several which when used in large amounts may kill fly larvæ but are placed in this class because of the large amount required or because of their extremely poisonous properties.

Iron sulphate has been used as a larvicide and in considerable amounts is stated to be effective. However, no studies of the effects of iron sulphate on the fertilizing value of manure have been reported. Our experiments indicate injury to the manure even from small applications of iron sulphate (see p. 10). Paris green and potassium cyanid are effective as larvicides, but are objectionable on account of their extremely poisonous nature.

UNSATISFACTORY OR PARTIALLY SATISFACTORY SUBSTANCES.

Kerosene emulsion.	Pyroligneous acid.
Kainit.	Sodium chlorid (table salt).
Isthmian Canal Commission larvicide.	Copper sulphate.
Iron sulphate.	Lime-sulphur mixture.
Several proprietary disinfectants.	Paris green.
Potassium cyanid.	Sodium fluorid.
Formaldehyde.	Ammoniacal gas liquor.
Calcium cyanamid.	

SATISFACTORY SUBSTANCES.

Borax.	Calcined colemanite.

By far the most effective, economical, and practical of the substances is borax in the commercial form in which it is available throughout the country.

Borax increases the water-soluble nitrogen, ammonia, and alkalinity of manure and apparently does not permanently injure the bacterial flora. The application of manure treated with borax at the rate of 0.62 pound per 8 bushels (10 cubic feet) to soil does not injure the plants thus far tested, although its cumulative effect, if any, has not been determined.

DIRECTIONS FOR TREATING MANURE WITH BORAX TO KILL FLY EGGS AND MAGGOTS.

Apply 0.62 pound borax or 0.75 pound calcined colemanite to every 10 cubic feet (8 bushels) of manure immediately on its removal from the barn. Apply the borax particularly around the outer edges of the pile with a flour sifter or any fine sieve, and sprinkle 2 or 3 gallons of water over the borax-treated manure.

The reason for applying the borax to the fresh manure immediately after its removal from the stable is that the flies lay their eggs on the fresh manure, and borax, when it comes in contact with the eggs, prevents their hatching. As the maggots congregate at the

outer edges of the pile, most of the borax should be applied there. The treatment should be repeated with each addition of fresh manure, but when the manure is kept in closed boxes less frequent applications will be sufficient. Where the calcined colemanite is available, it may be used at the rate of 0.75 pound per 10 cubic feet of manure, and is a cheaper means of killing the maggots. In addition to the application of borax to horse manure to kill fly larvæ, it may be applied in the same proportion to other manures, as well as to refuse and garbage. Borax may also be applied to floors and crevices in barns, stables, markets, etc., as well as to street sweepings, and water should be added as in the treatment of horse manure. After estimating the amount of material to be treated and weighing the necessary amount of borax a measure may be used which will hold the proper amount, thus avoiding subsequent weighings.

WARNING IN CONNECTION WITH THE USE OF BORAX-TREATED MANURE.

While it can be safely stated that no injurious action will follow the application of manure treated with borax at the rate of 0.62 pound for 8 bushels, or even larger amounts in the case of some plants, nevertheless borax-treated manure has not been studied in connection with the growth of all crops, nor has its cumulative effect been determined. It is therefore recommended that not more than 15 tons per acre of the borax-treated manure should be applied to the field. As truckmen use considerably more than this amount, it is suggested that all cars containing borax-treated manure be so marked, and that public-health officials stipulate in their directions for this treatment that not over 0.62 pound for 8 bushels of manure be used, as it has been shown that larger amounts of borax will injure most plants. It is also recommended that all public-health officials and others in recommending the borax treatment for killing fly eggs and maggots in manure warn the public against the injurious effects of large amounts of borax on the growth of plants.

COST OF BORAX TREATMENT.

The amount of manure from a horse varies with the straw or other bedding used, but 12 or 15 bushels per week represent the approximate amount obtained. As borax costs from 5 to 6 cents per pound in 100-pound lots in Washington, it will make the cost of the borax practically 1 cent per horse per day. And if calcined colemanite is purchased in large shipments the cost should be considerably less.

LITERATURE CITED.

American Public Health Association, Laboratory Section.
1912. Standard Methods for the Examination of Water and Sewage. 2d ed. New York, Amer. Pub. Health Assoc., 144 p. (Cover title: Standard Methods of Water Analysis. A. P. H. A. 1912.)

Folin, Otto, and Macallum, A. B.
1912. On the determination of ammonia in urine. *In* Jour. Biol. Chem., v. 11, no. 5, p. 523–525, June. See p. 523.

Haselhoff, E.
1913. Landwirtschaftlichen Versuchs. vols. 79 and 80, p. 399.
1913. Uber die Einwirkung von Borverbindungen auf das Pflanzenwachstum. *In* Landw. Vers-Stat., Bd. 79 u. 80, p. 399–429, pl. 4–7, 1913.

Herms, W. B.
1910. How to control the common house fly. Cal. State Board of Health Bul., May 5, no. 2, p. 269–277.

Howard, L. O.
1911. The House Fly—Disease Carrier. New York, Frederick A. Stokes Co., 312 p., illus. See p. 196 and 197.

Hutchison, R. H.
1914. The migratory habit of house-fly larvæ as indicating a favorable remedial measure, An account of progress. U. S. Dept. Agr., Bul. 14. 11 p. Feb. 28.

Russell, E. J., and Buddin, Walter.
1913. The action of antiseptics in increasing the growth of crops in soil. *In* Jour. Soc. Chem. Ind., v. 32, No. 24, p. 1136–1142, Dec. 31.

Smith, R. I.
1912. The house fly (*Musca domestica*). No. Car. Agr. Expt. Sta., Col. Agr. & Mech. Arts, Ann. Rpt. 34, p. 62–69, figs. 13–14. See p. 64.

Sörensen, S. P. L.
1907. Enzymstudien. *In* Biochem. Ztschr. Bd. 7, Hft. 1/2, p. 45–101, Dec. 6.

Van Slyke, D. D.
1911. A method for quantitative determination of aliphatic amino groups, Applications to the study of proteolysis and proteolytic products. *In* Jour. Biol. Chem., v. 9, No. 3/4, p. 185–207, May.

Wiley, Harvey W., editor.
1908. Official and provisional methods of analysis, Association of Official Agricultural Chemists. U. S. Dept. Agr., Bur. Chem., Bul. 107 rev. 272 p.

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